

Salmonella Prevalence in Market-Age Turkeys On-Farm and at Slaughter¹

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ABSTRACT The objective of this study was to compare the prevalence of *Salmonella* in market-age turkeys on-farm and at slaughter (i.e., before and after feed withdrawal, catching, loading, transportation, and preslaughter holding). Thirty birds were randomly selected from each of 6 commercial turkey flocks scheduled to be loaded and shipped to the abattoir during the evening of the same day. Selected birds were euthanized on the farm, and the cloacal contents, large intestine, crop, ceca, liver and gallbladder, and spleen were aseptically collected. At the abattoir, 30 birds from the same flock were randomly selected from the slaughter line, and the crop, ceca, liver and gallbladder, and spleen were collected for subsequent culture at the laboratory. All flocks studied were positive

for *Salmonella* at slaughter. No statistical difference was found between the overall prevalence on-farm and at slaughter. At both sampling points, the overall prevalence found was 33.3%. Diverging prevalence estimates were obtained based on the different sample types collected on-farm and at slaughter. In both cases, cecal content samples had the highest relative sensitivity (73.3% on-farm and 68.3% at slaughter). This study demonstrates that the preslaughter practices of feed withdrawal, catching, loading, transportation, and holding do not significantly alter the prevalence of *Salmonella* in market-age turkeys. Therefore, our results suggest that it may be possible to monitor the *Salmonella* status of turkey production farms based on samples collected at the abattoir.

Key words: turkeys, *Salmonella*, food safety, epidemiology

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INTRODUCTION

Foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year (Mead et al., 1999). *Salmonella* is the second most common cause of bacterial foodborne diseases, and poultry products are implicated as a major source of human foodborne salmonellosis. During slaughter and processing, *Salmonella* from the gastrointestinal tract of carrier birds can contaminate carcasses and the slaughter and processing line. Although efforts have concentrated primarily on controlling contamination within the abattoirs, a high proportion of carcasses are still found to be contaminated with *Salmonella*. According to Eblen et al. (2005), 19.6% of the turkey carcasses were contaminated with *Salmonella* during 1997 and 1998, compared with 1.2 and 6.9% for cattle and swine carcasses, respectively. The extent to which carcasses are contaminated is dependent upon the hygiene of the slaughter

and processing as well as the degree of carriage in the live birds entering the abattoir. However, we still do not have a clear understanding of *Salmonella* epidemiology in the preharvest turkey production system, and most of all, little is known about the factors affecting the prevalence of *Salmonella* in market-age turkeys. As a consequence, effective preharvest control measures to reduce the occurrence of this pathogen from the turkey supply are still lacking.

Most of the information on *Salmonella* in poultry available to date derives from studies conducted on broiler chickens, with very limited information available on the epidemiology of *Salmonella* in turkeys. Stern et al. (1995) and Whyte et al. (2001) demonstrated that transport increased the isolation rate of *Campylobacter* from broiler chickens. Likewise, the isolation rate of *Salmonella* from broiler chickens has been reported to increase after subjecting the birds to the stressful preslaughter practices of feed withdrawal and transportation (Line et al., 1997; Ramirez et al., 1997; Corrier et al., 1999). Such events can result in birds entering the slaughter and processing line with considerably higher contamination levels, serving as a significant source of cross-contamination for other birds and flocks and the slaughter and processing environment. However, there is a lack of knowledge concerning sources of *Salmonella* contamination for market-age turkeys.

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Table 1. *Salmonella* prevalence in 6 flocks of market-age turkeys, based on different sample types collected on-farm (n = 30 birds/flock)

Flock	Cloacal contents (%)	Large intestine (%)	Cecal contents (%)	Crop (%)	Spleen (%)	Liver and gallbladder (%)	Any sample ¹ (%)
A	3.3	0	6.7	3.3	0	ND ²	13.3
B	33.3	53.3	36.7	10	46.7	10	83.3
C	37.9	65.5	96.7	43.3	33.3	6.7	96.7
D	0	0	0	0	0	0	0
E	0	0	6.7	0	0	0	6.7
F	0	0	0	0	0	0	0
Overall	22/179 (12.3%) ^{acd}	35/179 (19.6%) ^{abd}	44/180 (24.4%) ^{bf}	17/180 (9.4%) ^{cd}	27/180 (15%) ^{cd}	5/150 (3.3%) ^e	60/180 (33.3%) ^f

^{a-f}Statistical comparison of proportions. Different superscript letters indicate $P < 0.05$.

¹Any sample = *Salmonella enterica*-positive birds in at least 1 of the samples collected.

²ND = not done.

Therefore, this study was conducted with the objective of determining if the preslaughter practices of feed withdrawal, catching, loading, transportation, and holding at the abattoir affect the prevalence of *Salmonella* in market-age turkeys. Additionally, the study aimed to measure the relative sensitivity of different sample types to estimate *Salmonella* prevalence in turkeys.

MATERIALS AND METHODS

Sampling

Six commercial tom turkey production flocks were selected for this study. All flocks were in the Midwest and delivered birds to the same abattoir. In each of the 6 flocks, 30 birds were randomly selected for on-farm sampling from a production barn scheduled to be shipped to the abattoir later on the same day (sampling occurred in the morning, approximately 10 h before shipping the birds to the abattoir). Birds were humanely euthanized by lethal injection (Sleep-Away, Fort Dodge Animal Health, Fort Dodge, IA) and necropsied. Individual samples were collected (cloacal contents, large intestine, ceca, crop, liver and gallbladder, and spleen), placed in individual sterile plastic bags, and transported to the laboratory for processing on the same day. Cloacal contents were collected by inserting a sterile plastic loop into the cloaca and, subsequently, removing fecal material from the loop (approximately 0.5 to 1 g).

Following feed withdrawal (8 to 10 h), catching, loading, transportation, and preslaughter holding (on the transport trailer), 30 birds from the same flock sampled on-farm were randomly selected on the slaughter line for sampling. Individual samples collected at slaughter included the following: crop, ceca, liver and gallbladder, and spleen. Samples were individually placed in sterile plastic bags and transported to the laboratory for processing on the same day.

The sample size (i.e., n = 30 birds/flock) was defined to allow estimation of the prevalence of positive birds at the 95% confidence level, with 10 to 15% precision of the estimate and with an expected prevalence of 20 to 30%, based on previous reports (Cox et al., 2000; Nayak et al., 2003). The experiment was repeated in 6 flocks, allowing

the total comparison of 180 market-age turkeys on-farm and at slaughter.

Bacteriological Procedures

At the laboratory, each sample (1 g) was processed for the isolation and identification of *Salmonella*, using 2 conventional isolation methods. Method 1 consisted of preenrichment (1:10) in buffered peptone water (24 h at 37°C, Difco Laboratories, Detroit, MI), followed by enrichment (1:100) in 9.9 mL of Rappaport-Vassiliadis broth (Difco Laboratories) with 20 µg/mL of novobiocin (24 h at 42°C, Sigma Chemical Co., St. Louis, MO) and an additional enrichment (1:100) in 9.9 mL of Rappaport-Vassiliadis broth (24 h at 42°C, Difco Laboratories). Method 2 consisted of primary enrichment (1:10) in tetrathionate broth (24 h at 37°C, Difco Laboratories), followed by secondary enrichment (1:100) in 9.9 mL of Rappaport-Vassiliadis broth (Difco Laboratories) with 20 µg/mL of novobiocin (24 h at 42°C, Sigma Chemical Co.) and an additional enrichment (1:100) in 9.9 mL of Rappaport-Vassiliadis broth (24 h at 42°C, Difco Laboratories). From the last enrichment (for each isolation method), aliquots were plated on Xyl-Lysine Tergitol-4 (Difco Laboratories) and brilliant green sulfa agars (24 h at 37°C, Difco Laboratories). Suspect colonies were individually picked and streaked on Rambach agar plates (24 h at 37°C, DRG International Inc., Mountainside, NJ) for confirmation (Gruenewald et al., 1991).

Statistical Analysis

Salmonella prevalence for each flock was estimated based on each sample type collected and overall (i.e., based on the combination of the results from multiple samples from each individual bird). Relative sensitivity for each sample type collected and agreement (κ statistic) between different sample types were determined according to Thrusfield (1995). Proportions were compared by χ^2 , and $P < 0.05$ was applied for statistical inferences.

RESULTS AND DISCUSSION

Feed withdrawal, catching, loading, transportation, and holding at the abattoir constitute common preslaughter

Table 2. *Salmonella* prevalence in 6 flocks of market-age turkeys, based on different sample types collected at slaughter (n = 30 birds/flock)

Flock	Cecal contents (%)	Crop (%)	Spleen (%)	Liver and gallbladder (%)	Any sample ¹ (%)
A	10	0	0	ND ²	10
B	36.7	14.3	33.3	45.5	73.3
C	66.7	10	20	13.3	80
D	10	0	0	16.7	23.3
E	6.7	3.3	0	3.3	6.7
F	6.7	0	0	0	6.7
Overall	41/180 (22.8%) ^a	8/178 (4.5%) ^b	16/178 (9%) ^{bc}	20/142 (14.1%) ^{ac}	60/180 (33.3%) ^d

^{a-d}Statistical comparison of proportions. Different superscript letters indicate $P < 0.05$.

¹Any sample = *Salmonella enterica*-positive birds in at least 1 of the samples collected.

²ND = not done.

ter practices in the poultry industry. However, although necessary, these practices are also considered to be important causes of stress for the birds. It has been shown that exposure to stressors increases transmission, colonization, and shedding (i.e., releasing of bacteria into the environment) of *Salmonella* in chickens (Holt and Porter, 1992; Holt et al., 1995, 1998). Particularly, the effect of feed withdrawal and transportation on foodborne pathogens (*Campylobacter* and *Salmonella*) has been extensively investigated in broiler chickens (Rigby and Pettit, 1980, 1981; Stern et al., 1995; Line et al., 1997; Ramirez et al., 1997; Byrd et al., 1998; Corrier et al., 1999; Whyte et al., 2001; Northcutt et al., 2003). In turkeys, however, almost no information is available. Recently, Wesley et al. (2005) investigated the effect of preslaughter events on the prevalence of *Campylobacter jejuni* and *Campylobacter coli* in market-age turkeys, and they reported that a shift in the dominance of *Campylobacter* species occurs in the intestinal tract of the birds. However, it is still not known if any effect occurs on *Salmonella*. Therefore, this study focused on the investigation of the potential effect of preslaughter practices on *Salmonella* prevalence at slaughter in market-age turkeys.

Prevalence estimates based on samples collected on-farm and at slaughter are presented in Tables 1 and 2, respectively. The overall prevalence, based on any sample type (i.e., birds positive in at least 1 of the samples collected), was the same (33.3%) for both sampling points (i.e., on-farm and at slaughter). For individual flocks, no difference was found between the 2 sampling points for flocks A, E, and F, whereas a significant decrease ($P < 0.05$) in prevalence was found for flocks B and C. A significant increase ($P < 0.05$) was found only for flock D (Figure 1). The absence of an effect of the preslaughter practices on *Salmonella* prevalence in market-age turkeys found in this study contrasts with the effect reported in broiler chickens (Line et al., 1997; Ramirez et al., 1997; Corrier et al., 1999). A possible explanation for this divergence may be based on the age of the birds. Whereas broiler chickens are subjected to the preslaughter practices at approximately 6 to 8 wk of age, tom turkeys only reach market age when they are approximately 18 to 21 wk old. Recent studies have shown that the complexity of the intestinal bacterial

community, as well as the immune response against *Salmonella* infections in broiler chickens, increases with age (Van der Wielen et al., 2002; Lu et al., 2003; Beal et al., 2005). We hypothesize that an established and more complex bacterial community, as well as a higher level of immune resistance in the intestinal tract of older market-age turkeys, may create a hostile environment for the establishment of new pathogens, such as *Salmonella*, particularly when birds are exposed to low doses of the bacteria. An established, complex intestinal microbial community and a mature immune system could prevent a significant increase in the prevalence of *Salmonella* due to new infections, even during exposure of market-age turkeys to a set of stressors associated with the preslaughter practices of the poultry industry. This hypothesis is supported by reports on the successful administration of adult turkey intestinal contents to young poults in protecting against *Salmonella* challenges (Reid and Barnum, 1983; Seuna et al., 1985).

As expected, different prevalence estimates were obtained based on the different sample types collected at both sample points (i.e., on-farm and at slaughter). Cecal content samples had the highest relative sensitivity in both samplings (73.3 and 68.3%, Table 3). The observation of a higher frequency of *Salmonella* in the ceca than in

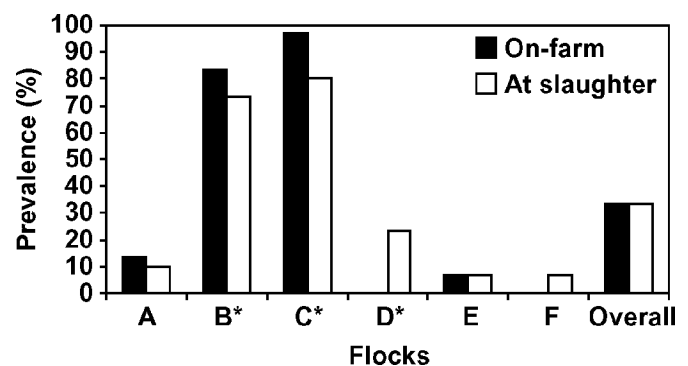


Figure 1. Prevalence of *Salmonella* in 6 market-age turkey flocks, on-farm and at slaughter. Prevalence estimates are based on the proportion of birds positive in at least 1 of the individual samples collected. Asterisks (*) indicate a significant difference ($P < 0.05$) between on-farm and at-slaughter prevalence estimates.

Table 3. Relative sensitivity of different sample types collected on-farm and at slaughter to estimate *Salmonella* prevalence in market-age turkeys

Sample type	On-farm (%)	At slaughter (%)
Cloacal contents	36.7	—
Large intestine	59.3	—
Cecal contents	73.3	68.3
Crop	28.3	13.6
Spleen	45	26.7
Liver and gallbladder	8.9	37.7

any other portion of the digestive system is in agreement with the findings of Fanelli et al. (1971) and Barrow et al. (1988), who investigated the occurrence of *Salmonella* in the different compartments of the digestive system of chickens. Yamamoto et al. (1961) also reported a higher isolation frequency of *Salmonella* from the ceca than from other segments of the intestine of market-age turkeys experimentally infected, which supports our observations under natural infection conditions.

Cloacal content (i.e., feces from the intestinal tract and urates from the kidneys) sampling was included in this study due to its potential application as a convenient tool to monitor the *Salmonella* status of live birds (i.e., on-farm). However, this study showed that cloacal content samples were not efficient in identifying positive birds with a relative sensitivity of only 36.7% (Table 3). The agreement between this sample type and cecal contents in identifying positive birds was moderate (49.3%). A portion of the large intestine was also collected to further compare the efficiency of cloacal content samples. The on-farm *Salmonella* prevalence estimates (Table 1), based on large intestine and cloacal content samples, were similar (19.6 and 12.3%, respectively). However, the agreement between both sample types was only moderate (44.3%).

A potential increase of the occurrence of *Salmonella* bacteremia (due to the stress associated with the pre-slaughter practices) was also investigated by monitoring changes in the isolation frequency of the bacteria from spleen and liver and gallbladder. Overall, no difference was found between the isolation rates of *Salmonella* from spleen samples collected on-farm (15%) and at slaughter (9%). However, a significant increase ($P < 0.05$) in the *Salmonella* isolation rate was found based on liver and gallbladder samples (3.3% on-farm vs. 14.1% at slaughter). Based on this observation, we hypothesize that the stress caused by the preslaughter practices favors the dissemination of *Salmonella* within the birds, which may result from an effect on the immune system or from an increase in virulence of the bacteria. The role of the neuro-endocrine environment, particularly in the intestinal tract, in the pathogenesis of enteric bacterial infections is increasingly being recognized. It has been demonstrated (in vitro) that intestinal pathogens express virulence determinants in response to environmental signals indicating host stress (Alverdy et al., 2000). However, little is known about the effect of catecholamines released into the intestine on the carriage and virulence of bacterial

pathogens in vivo (Vlisidou et al., 2004). Therefore, further studies are necessary to determine the validity of the hypothesis presented here.

Many believe that the prevalence of *Salmonella*-positive birds at slaughter will increase as a result of the stress associated with the preslaughter practices, which may lead to recrudescence of latent infections. Also, a potential increase in prevalence may occur due to the rapid transmission of *Salmonella* during transportation or in the pre-slaughter holding period, as demonstrated to occur in pigs (Hurd et al., 2001; Rostagno et al., 2003). Whatever the case, this study shows that, overall, it is not a common occurrence in flocks of market-age turkeys. However, although there was no prevalence increase from the production farms to the abattoir, our results indicate that turkey flocks still pose a significant food safety risk, based on the relatively high on-farm and abattoir prevalence found. Our results are aligned with the high *Salmonella* prevalence in turkey carcasses (19.6%), reported recently by Eblen et al. (2005).

Important aspects to be considered in *Salmonella* monitoring are the type of sample and the time of sampling to determine the infection status of flocks with the highest sensitivity. In this study, in addition to demonstrating that the common preslaughter practices of feed withdrawal, catching, loading, transportation, and holding do not affect *Salmonella* prevalence at slaughter in market-age turkeys, we determined that cecal contents constitute the sample of choice to estimate *Salmonella* prevalence in market-age turkeys, on-farm and at slaughter. Furthermore, this study shows that the convenient sampling of birds at slaughter can be applied to infer about on-farm *Salmonella* prevalence in turkeys.

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